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Optimizing Productivity of Food Crop Genotypes in Low Nutrient Soils

Prepared by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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BIOLOGICAL NITROGEN FIXATION EFFICIENCY IN BRAZILIAN COMMON BEAN GENOTYPES AS MEASURED BY ^{15}N METHODOLOGY

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Abstract

Common bean (*Phaseolus vulgaris* L.) represents the main source of protein for the Brazilian and other Latin-American populations. Unlike soybean, which is very efficient in fixing atmospheric N_2 symbiotically, common bean does not dispense with the need for N fertilizer application, as the biologically fixed N (BNF) seems incapable to supplement the total N required by the crop. A experiment under controlled conditions was conducted in Piracicaba, Brazil, to assess N_2 fixation of 25 genotypes of common bean (*Phaseolus vulgaris* L.). BNF was measured by ^{15}N isotope dilution using a non-N_2 fixing bean genotype as a reference crop. The common bean genotypes were grown in low (2.2 mg N kg^{-1} soil) or high N content soil (200 mg N kg^{-1} soil), through N fertilizer application, as urea-^{15}N (31.20 and 1.4 atom % ^{15}N, respectively). The bean seeds were inoculated with *Rhizobium tropici* CIAT 899 strain and the plants were harvested at grain maturity stage. The contribution of BNF was on average 75% of total plant N content, and there were differences in N fixing capacity among the bean genotypes. The most efficient genotypes were Horizonte, Roxo 90, Grafite, Aporé and Vereda, when grown in high N soil. None of the genotypes grown in low N soil was efficient in producing grains compared to those grown in high N soil, and therefore the BNF was not able to supply the total N demand of the bean crop.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the main source of protein for the Brazilian population. Nitrogen (N) is the nutrient taken up in larger amounts by the bean plant, and N supplied as fertilizer is expensive and easily lost by volatilization or leaching [1, 2]. Approximately 50% of total N uptake is exported in the grain and the remainder stays in the soil in the form of crop residues [1, 3].

Maximizing the use of N by bean is important because of the economic and environmental aspects, as this nutrient presents risk to the environment as potentially contaminating groundwater [4] due to leaching of nitrate. Moreover, it is observed in field that it is possible to achieve bean yields above 2500 kg ha^{-1} according to the BNF process without N addition [5, 6]. However, inoculants are used in only 2–3% of the acreage of common bean [7]. As the ability for nodulation varies with the common bean genotypes and

Rhizobium strain, the nodulation efficiency is highly dependent on the genotype of common bean [8].

The objective of this study was to compare the common bean's ability for effective BNF, evaluated using the ^{15}N dilution technique, for 25 common bean genotypes grown under controlled conditions with no limitation to BNF (light, temperature, water, nutrients, and efficient rhizobium strain association with common bean).

2. MATERIAL AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at the Center for Nuclear Energy in Agriculture (CENA / USP), located at latitude 22°42'30" S, longitude 47°38'01" W and 554 m altitude, in Piracicaba, Sao Paulo, Brazil.

The study were performed in 3.0 l plastic pots, containing 2.5 kg of air-dried soil, collected from the 0 to 0.20 m layer of a dystrophic Typic Haplustox [9]. The soil had 280, 70 and 650 g kg^{-1}content of clay, silt and sand, respectively, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$), 4.5; organic matter, 18.0 g dm^{-3}; P extracted by resin, 5 mg dm^{-3}; K, 0.6 $mmol_c$ dm^{-3}; Ca, 11.5 $mmol_c$ dm^{-3}; Mg, 5.2 $mmol_c$ dm^{-3}; H + Al, 35.4 $mmol_c$ dm^{-3}; CEC, 52.7 $mmol_c$ dm^{-3}; sum of bases, 17.3 $mmol_c$ dm^{-3}; base saturation, 32.8%, according to methodology described by [10] and P by Mehlich-1, 3 mg dm^{-3} [11].

After application of lime (calcium carbonate equivalent = 110%) to raise the base saturation to 70% for the common bean, according to the official recommendation of the Bulletin 100 [12], the soil was incubated for 30 days and the moisture content was maintained at approximately 70% of water holding capacity.

The experimental design was a randomized complete block with four replications. The experiment consisted of 52 treatments, which were divided into two groups. Each group had 26 treatments, which consisted of 25 common bean genotypes (Aporé, BRS Grafite, BRS Horizonte, BRS Pitanga, BRS Vereda, BRSMG Pioneiro, Carioca 80, CNF 10, FT Nobre, IAC Tybatã, IAC UNA, Jalo precoce, LP 01-38, Ônix, Ouro Negro, Pontal, Rosinha, Roxão EEP, Roxinho, Roxo 90, Rubi, Rudá, Sangue de Boi, Thayú e Timbó) and a control plant (non-nodulating common bean).

The first treatment (group of 25 common bean genotypes and one standard plant species) was carried out to compare the BNF between genotypes in the absence of mineral-N. As the ^{15}N isotopic dilution method requires the labeling of soil N, a low rate of mineral N (2.20 mg kg^{-1} soil as ^{15}N-urea with high enrichment of 31.20 atom % ^{15}N was used. The second treatment was to compare the BNF by the same common bean genotypes used in the first group in the presence of mineral-N. N was applied at 200 mg kg^{-1} soil as ^{15}N-urea (1.41 atom % ^{15}N). To avoid inhibiting the nodulation of plants, the mineral N dose was split in two applications, 1/3 at sowing and the remaining portion at the V4 stage (issuance of the third trifoliate leaf).

The P and K fertilization was performed with application of 200 mg kg^{-1} soil of P as triple superphosphate and 200 mg kg^{-1} soil of K as potassium sulfate in all pots. Micronutrient fertilization was performed by application of a nutrient solution in all treatments at rates of 0.5 mg kg^{-1} of B, 1.5 mg kg^{-1} of Cu, 3.0 mg kg^{-1} of Fe, 2.0 mg kg^{-1} of Mn, 3.0 mg kg^{-1} of Zn and 0.1 mg kg^{-1} of Mo.

The experiment was conducted in summer under conditions of high intensity of light and temperature, in order not to limit the BNF. The seeds of the bean genotypes were inoculated with the strain of *Rhizobium tropici* CIAT 899 (SEMIA 4077), which is recommended commercially for common bean in Brazil. The inoculation was done three hours before sowing, at a rate of 500 grams of inoculants (109 viable cells g^{-1} peat) to 50 kg of seed plus 300 ml of 10% sugar solution (w: v) to improve its adherence to the seeds.

Five common bean seeds from each genotype and standard plant (non-nodulating bean genotype - NORH 54) were sown in each experimental, thinned to one plant per pot. Soil moisture was maintained at approximately 70% of water retention capacity during the experiment. Plants were harvested after the pod maturity stage and separated into roots, shoots (stems, leaves and bark of legumes) and grain. At harvest, the number of nodules per plant and fresh weight of nodules per plant were measured.

Plant samples were dried and ground in Wiley-type mill, passed through 10 mesh sieve and weighed on a precision analytical balance (five decimal) for ^{15}N isotope (atom %) and total N determination in a mass spectrometer (IRMS) interfaced with an elemental analyzer, according to methodology described by [13].

2.1. N calculations

N uptake was calculated according to the following equation:

$$TN = N \times Sdm$$

Where:

TN = plant total N content (mg $plant^{-1}$);
N = plant N concentration (g kg^{-1});
Sdm = shoot dry mater (g $plant^{-1}$).

The equation of symbiotic fixed N is presented below [14].

$$\%\text{Npdfix} = 1 - \left[\frac{(\%A^{15}Nexcess)_{genotype}}{(\%A^{15}Nexcess)_{control}} \right]$$

Where:

% Npdfix: % N in the plant derived from the symbiotic fixation;
$(\%A\ ^{15}N\ \text{excess})_{genotype}$: atom % ^{15}N excess in the nodulated common bean genotype;
$(\%A\ ^{15}N\ \text{excess})_{control}$: atom % ^{15}N excess in the control plant (non-nodulating bean);

With the total N content in the SDM of common bean genotypes (TN, mg $plant^{-1}$), the amount of N in the plant derived from symbiotic fixation (QNpdfix) was calculated.

$$\text{QNpdfix} = \frac{(\%\text{Npdfix}) . (\text{NA})_{genotype}.}{100}$$

Where:

QNpdfix: amount of N in the plant derived from symbiotic fixation (mg);
% Npdfix: % of N in the plant derived from symbiotic fixation;
$(TN)_{genotype}$: total N content in the Sdm of the common bean genotype (mg).

2.2. Statistical analysis

Cluster analysis of common bean genotypes was carried out with the SAS 9.1 - “Statistical Analysis System” [15] and SYSTAT version 10.2 software programs, using the UPGMA (unweighted pair group arithmetic average clustering). The cluster analysis was preceded by the standardization of data before the Euclidian distances calculation, as the studied variables presented different scales. After standardization all the variables were equally important in the determination of these distances. Final results of the groups were presented as dendrograms. Within the text, the symbol * preceding a genotype denotes the treatment fertilized with the higher rate of urea.

3. RESULTS AND DISCUSSION

With high urea application, the number of nodules = 0; shoot DM = 54.24 g plant^{-1}; N uptake in shoots = 307 mg plant^{-1}; N derived from fertilizer in shoots = 69.0%; N derived from BNF in shoots = 0%; N derived from soil in shoots = 31.0%; Recovery of urea N in shoots = 42.4%; Root DM = 15.76 g plant^{-1}; Grain DM = 3.83 g plant^{-1}; N uptake in grain = 141 mg plant^{-1}; number of pods = 20.5; number of grains = 40.3; N derived from fertilizer in grain = 69.4%; N derived from BNF in grain = 0%; N derived from soil in grain = 30.6%; Recovery of urea-N in grain = 19.7%; total shoot DM = Shoot DM + Grain DM = 58.07 g plant^{-1}; N uptake by total shoot DM = 449 mg plant^{-1}; N derived from BNF in total shoot DM = 0%; N derived from fertilizer in total shoot DM = 69.1%; Recovery of urea-N in total shoot DM = 62.1%; and N derived from soil in total shoot DM = 30.9%.

With low urea application, the number of nodules = 0; shoot DM = 12.40 g plant^{-1}; N uptake in shoots = 80.5 mg plant^{-1}; N derived from fertilizer in shoots = 2.5%; N derived from BNF in shoots = 0%; N derived from soil in shoots = 97.5%; Recovery of urea N in shoots = 36.4%; Root DM = 5.37 g plant^{-1}; Grain DM = 0.96 g plant^{-1}; N uptake in grain = 31.6 mg plant^{-1}; number of pods = 9.0; number of grains = 16,5; N derived from fertilizer in grain = 1.6%; N derived from BNF in grain = 0%; N derived from soil in grain = 98.4%; Recovery of urea-N in grain = 9.4%; total shoot DM = Shoot DM + Grain DM = 13.36 g plant^{-1}; N uptake by total shoot DM = 112 mg plant^{-1}; N derived from BNF in total shoot DM = 0%; N derived from fertilizer in total shoot DM = 0.5%; Recovery of urea-N in total shoot DM = 45.9%; and N derived from soil in total shoot DM = 99.5%.

The N uptake from BNF in total shoot DM of 25 common bean genotypes at low or high application of urea, correlated significantly and positively with shoot DM (0.656 ***), grain DM (0.699 ***) and root DM (0.493 ***). This increase in production of DM of shoots, roots and grain indicates that plants responded both in grain production as well as shoots and roots biomass to N supplied by the BNF. Considering these three variables, the level of homogeneity of 1.0 in the Euclidean distance, we observed the formation of the following seven distinct homogeneous groups as shown in Fig. 1.

1st: Jalo Precoce, Grafite, Roxão EEP and Rubi;
2nd: *Roxão EEP and *Jalo Precoce;
3rd: Sangue de Boi, CNF 10, Ônix, Pontal, Roxo 90, Horizonte, Thayú, Pitanga, IAC UNA, FT Nobre, Aporé, LP 01-38, Ouro Negro, Pioneiro, Rudá, Carioca 80, Rosinha, Tybatã, Roxinho and Vereda;
4th: Timbó;
5th: *Carioca 80, *FT Nobre, *Roxo 90, *Ouro Negro, *Rosinha, *Pontal, *Pioneiro, *CNF 10, *Rudá, *Rubi, *Pitanga, *Aporé, *Horizonte, *Grafite, *Sangue de Boi, *LP01-38, *Ônix and *Tybatã;

6th: Vereda, *Roxinho, *IAC UNA and *Thayú; and
7th: *Timbó.

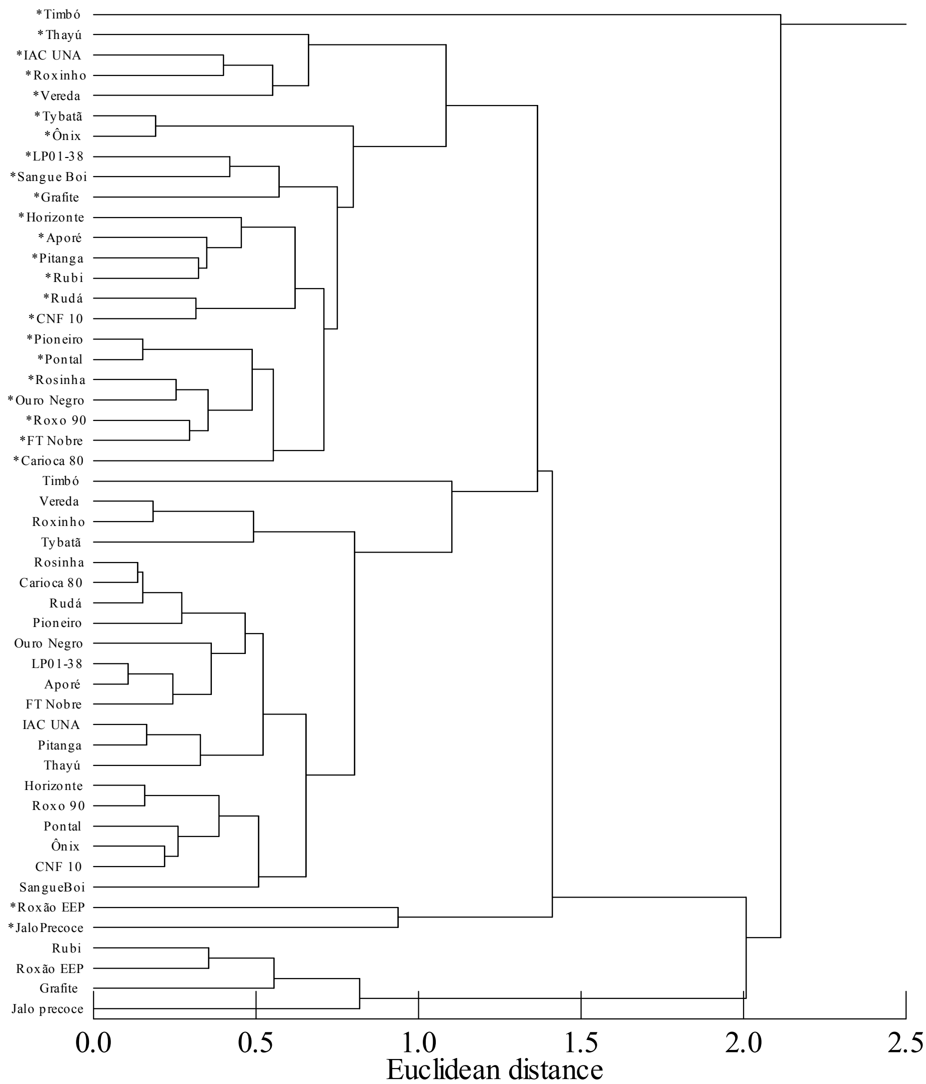

*FIG. 1. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean, based on DM (g) of shoots, roots and grain and N in total shoot DM derived from BNF. * denotes genotypes that received high urea fertilization.*

There was a response in grain production, and shoot and root DM to BNF, even in genotypes fertilized with the higher rate of urea, and this productive response varied among genotypes (Fig. 1). The genotypes, in association with rhizobium, which most benefited from BNF and produced more DM of shoots roots and grain were Vereda, Roxinho, IAC UNA and Thayú, in the treatment with higher rate of N fertilization (6th group). The BNF contributed around 70% of total N in these genotypes, even with the higher addition of urea-N; it shows that, depending on the bean genotype, BNF can contribute with high amounts of N_2 fixation, even in N fertilized plants. Moreover, considering the seven groups of genotypes, there was no genotype in the treatments with low and high fertilizer N classified in the same group. Therefore, the genotypes that depended only on soil N and BNF as N sources for development were not as efficient in terms of shoot, root and grain DM production, as those receiving a higher rate of N fertilizer. On average, considering the 25 bean genotypes, fertilized with low or high urea, BNF contributed approximately 75% of total N absorbed by the plants.

The number of plant nodules correlated positively with the fresh weight of nodules (0.704 ***), the total N content in shoot DM (0.367***) and with the total plant N derived from BNF (0.350***). Although significant, the correlation coefficients of the number and fresh weight of nodules with total plant N and N uptake in the plant from BNF were relatively low. The number and fresh weight of nodules had coefficients of variation of 18.2 and 20.2%, respectively (Table 1).

TABLE 1. NODULE FRESH WEIGHT AND NUMBER, N UPTAKE OF SHOOTS AND N DERIVED FROM BNF OF 25 GENOTYPES OF COMMON BEAN AT LOW (+) OR HIGH (++) UREA APPLICATION

Genotype	Nodule fresh weight (mg plant^{-1})		Nodule number		N uptake (mg)			
					Total in shoots		Derived from BNF	
	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea
Carioca 80	0.59	0.52	162	173	968	1412	855	1048
Rudá	1.03	0.66	171	107	955	1271	840	859
Aporé	1.34	2.22	139	227	853	1405	755	914
Pontal	1.82	1.28	195	199	855	1358	729	885
Horizonte	1.83	3.09	191	242	844	1403	720	907
Pioneiro	0.76	0.95	114	130	909	1401	792	889
Rosinha	0.84	1.05	155	212	954	1358	854	909
Rubi	0.95	1.62	136	163	680	1321	565	828
Vereda	1.51	2.11	173	248	1158	1560	1028	1129
Tybatã	1.40	1.53	158	155	944	1456	837	936
CNF 10	1.04	0.81	157	145	894	1276	771	742
Roxão EEP	1.03	0.97	141	113	571	1005	446	539
Sangue de Boi	0.84	0.73	126	115	700	1332	584	838
Roxinho	1.25	0.86	135	135	1096	1628	967	1097
Timbó	0.66	0.68	108	102	1048	1387	935	969
Roxo 90	1.49	2.02	147	301	837	1399	723	906
Pitanga	1.47	1.43	158	149	1050	1369	928	925
Ouro Negro	1.53	1.30	171	149	969	1424	838	905
Ônix	1.41	1.08	142	137	851	1415	736	944
IAC UNA	0.80	1.06	153	168	1045	1628	888	1159
Jalo precoce	0.77	1.19	133	171	404	1238	256	678
Grafite	1.21	1.93	139	246	540	1251	433	834
Thayú	1.27	1.72	157	268	1128	1821	1006	1351
FT Nobre	1.36	1.14	124	145	930	1532	772	1015
LP01-38	1.45	1.56	154	157	873	1273	740	741
Average	1.26		162		1140		839	
CV (%)	20.2		18.2		12.7		13.6	

Considering these four variables, the level of homogeneity in the Euclidean distance of 1.0 (Fig. 2), seven homogeneous groups were obtained:

1st: Jalo Precoce, Grafite, Roxão EEP, Rubi, Sangue de Boi and *Roxão EEP;
2nd: Horizonte and Pontal;
3rd: Pitanga, Tybatã, Ouro Negro, LP01-38, Roxo 90, Ônix, Aporé, FT Nobre, CNF 10, Rudá, IAC UNA, Rosinha, Carioca 80, *CNF 10, *Jalo Precoce, *Rosinha, *Pontal, *LP01-38, *Rubi, Roxinho, Thayú, Vereda, *Tybatã, *Pitanga, *Ouro Negro, *FT Nobre, *Ônix, *Pioneiro, *Timbó, *Rudá, *Sangue de Boi, Timbó and Pioneiro;
4th: *Carioca 80, *Roxinho and *IAC UNA;
5th: *Thayú;
6th: *Roxo 90, *Grafite, *Aporé and *Vereda; and

7^{th}: *Horizonte.

Among these groups, the 6^{th} and 7^{th} groups, formed by the genotypes *Horizonte, *Roxo 90, *Grafite, *Aporé and *Vereda stood out, especially on nodulation and N accumulation (Table 1). These five common bean genotypes with highest nodulation were grown with the high N rate.

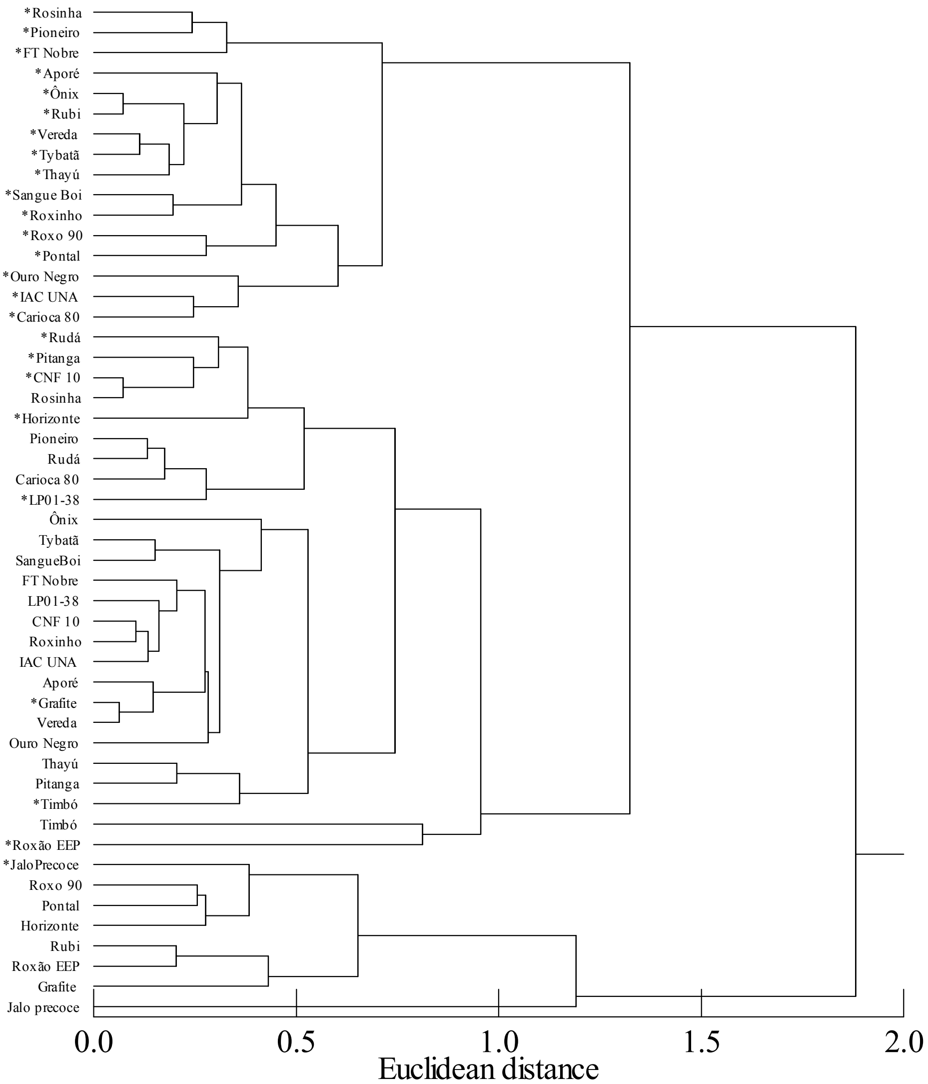

*FIG. 2. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean based on number and fresh weight of nodules, accumulation of N (mg) in total shoot dry matter and N in total shoot DM derived from BNF. * denotes genotypes that received high urea fertilization.*

Considering the edible portion and economic interest of the crop, it was observed that the grain DM of common bean genotypes, fertilized with a low or high rate of urea-N, correlated significantly and positively with the number of pods (0.824***) and the number of grains (0.878***). The number of pods and seeds per plant were highly and positively correlated with grain production. The number of pods is one of the most important in increasing the production of common bean [16].

1st: (low productive group) formed by treatment Jalo Precoce;
2nd: (moderately productive group) by treatments Grafite, Roxão EEP, Rubi, Horizonte, Pontal, Roxo 90 and *Jalo Precoce;
3rd: (moderately to very productive group) by treatments *Roxão EEP, Timbó, *Timbó, Pitanga, Thayú, Ouro Negro, Vereda, *Grafite, Aporé, IAC UNA, Roxinho, CNF 10, LP01-38, FT Nobre, Sangue de Boi, Tybatã, Ônix, *LP01-38, Carioca 80, Rudá, Pioneiro, *Horizonte, Rosinha, *CNF10, *Pitanga and *Rudá; and the
4th: (very productive group) by the treatments *Carioca 80, *IAC UNA, *Ouro Negro, *Pontal, *Roxo 90, *Roxinho, *Sangue de Boi, *Thayú, *Tybatã, *Vereda, *Rubi, *Onix, *Aporé, *FT Nobre, *Pioneiro and *Rosinha.

From grain DM, number of pods and number of grains (Table 2), in the level of homogeneity of 1.0 in the Euclidean distance dendrogram (Fig. 3), we observed the formation of four homogeneous groups:

TABLE 2. MEAN DRY MATTER YIELD OF SHOOTS, ROOTS AND GRAIN, NUMBER OF PODS AND GRAINS OF 25 GENOTYPES OF COMMON BEAN AT LOW (+) OR HIGH (++) UREA APPLICATION

Genotype	Dry matter yield (g plant^{-1})						Number plant^{-1}			
	Shoot		Root		Grain		Pods		Grain	
	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea
Carioca 80	20.0	29.4	7.3	9.6	28.3	32.7	20.0	24.3	108.0	122.3
Rudá	18.7	26.1	6.5	8.3	27.8	29.4	19.0	23.3	115.7	150.3
Aporé	17.6	35.5	5.4	11.1	24.5	28.1	19.0	27.7	95.7	172.7
Pontal	17.0	35.3	7.8	9.5	20.1	33.7	11.7	24.0	74.7	160.7
Horizonte	20.0	38.9	6.0	9.2	17.3	25.5	12.0	21.3	66.7	132.3
Pioneiro	18.8	34.7	5.3	8.8	28.4	35.2	20.0	31.3	121.3	174.3
Rosinha	20.8	34.5	6.6	11.3	29.3	35.9	22.7	32.3	127.7	189.0
Rubi	14.6	32.8	4.8	9.5	15.0	29.7	11.0	28.7	59.0	158.7
Vereda	23.7	42.8	10.6	16.4	24.8	30.1	18.0	27.0	105.7	160.0
Tybatã	21.2	45.2	9.3	12.4	22.4	29.5	17.7	26.3	104.0	154.7
CNF 10	16.8	26.3	7.2	8.0	23.4	28.9	16.0	22.7	94.3	131.7
Roxão EEP	13.2	27.2	5.3	10.8	13.3	21.0	11.7	26.7	50.7	117.7
Sangue de Boi	14.5	30.6	7.1	14.1	20.7	31.0	17.3	30.0	103.3	161.0
Roxinho	23.2	36.3	10.5	15.3	23.7	32.2	16.7	30.3	99.7	172.0
Timbó	31.9	43.8	9.3	21.2	15.7	19.8	22.3	21.7	81.7	100.3
Roxo 90	19.8	33.5	5.6	13.2	19.2	33.2	14.3	27.0	72.7	161.7
Pitanga	21.3	30.7	5.6	9.6	22.6	27.6	21.7	24.7	106.3	136.0
Ouro Negro	18.9	30.1	3.8	11.9	26.2	35.5	17.0	26.7	87.7	135.0
Ônix	19.6	41.9	6.9	12.2	21.8	28.8	13.3	28.7	94.3	159.3
IAC UNA	21.4	35.6	6.1	12.6	24.0	32.3	17.3	24.7	92.0	138.7
Jalo precoce	10.3	33.8	5.3	16.4	7.0	18.2	5.3	15.7	21.3	56.0
Grafite	14.8	35.4	8.1	14.6	10.4	24.9	8.7	18.3	48.0	102.0
Thayú	21.7	38.5	6.9	14.4	24.9	30.5	21.7	27.0	107.3	146.3
FT Nobre	19.6	33.4	5.4	12.7	21.9	33.5	15.7	29.3	93.0	187.3
LP01-38	17.9	30.9	4.7	11.9	24.5	29.2	15.3	18.7	93.0	99.0
Average	26.8		9.4		25.5		20.9		116.1	
CV (%)	11.9		15.2		12.9		13.7		14.5	

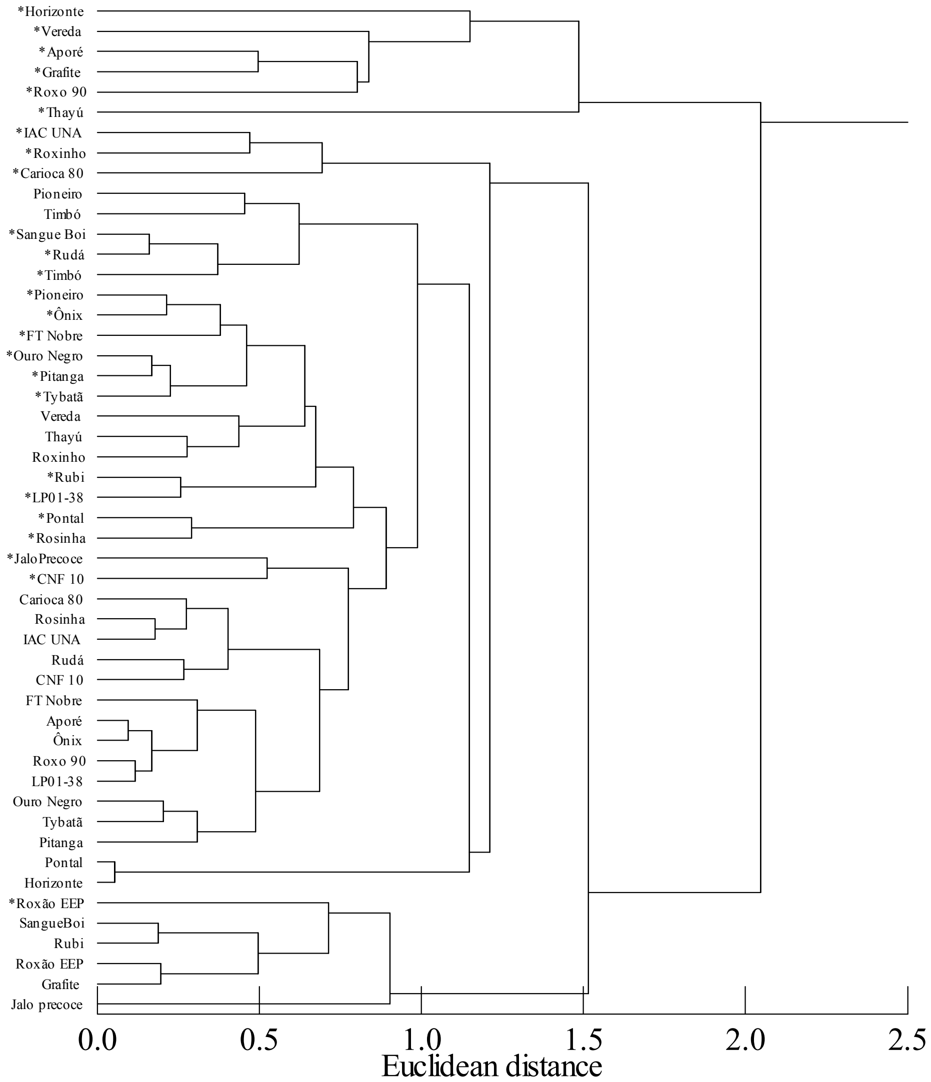

*FIG. 3. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean based on grain dry matter, number of pods and number of grains. * denotes genotypes that received high urea fertilization.*

Among the 25 common bean genotypes in the treatment with low N fertilizer, none were classified in the group of very grain productive. This indicates that BNF was not able to meet all the N demands of the plant N to achieve equivalent grain yields of the same genotypes that received urea-N fertilization. Common bean is considered to be a species with a low capacity for nodulation and BNF compared to other grain legumes [17]. Nevertheless, the BNF has contributed up to 90 kg–N ha^{-1} in various bean crops, which represented 40 to 50% of the demand of this crop [18]; in seven field experiments the observed average and maximum values were, respectively, 35 and 70% of N in the plant from the atmosphere in common bean genotypes [19].

The efficiency of bean BNF depends, among other factors, on the genotype. The selection of genotypes more efficient in symbiosis with rhizobia is an alternative to reduce N fertilization. For example, in field studies, fertilization with 20 kg–N ha^{-1}, together with inoculant strain of *R. tropici* CIAT 899 permitted common bean to yield higher than 3000 kg ha^{-1}, equivalent to the application of 160 kg–N ha^{-1} [6]. In addition, one must consider that the success of inoculation with strains of bean rhizobia with high efficiency is associated with competitive ability of such strains and adaptation to environmental conditions [20]. Under appropriate environmental conditions, the atmospheric N_2 fixed by symbiosis can meet most of the N needs of common bean [21]. However, some soil conditions such as low pH and high concentrations of Al often limit all stages of root infection, nodule formation and assimilation of N by the plant [22].

Studies under field conditions have shown that it is possible to achieve bean yields above 2500 kg ha^{-1} by the BNF process without addition of mineral N fertilizer [5, 6]. It is noteworthy, however, that the energy used in BNF is ATP; the photosynthates are important to the process of BNF for the N_2-fixing organisms, because they generate reducing power and ATP for the nitrogenase system, are substrates for growth and maintenance of microbial cells and supply carbon skeletons, ATP and reducing power for the assimilation of NH_3 [23]. Therefore, environmental conditions or management practices that influence the availability of photosynthates also affect BNF. The bean growing season (winter/summer), for example, influences the availability of photosynthates, and thus the BNF.

4. CONCLUSIONS

- Common bean genotypes differ in their ability to fix N_2 from the atmosphere through BNF.

- Among 25 common genotypes, Horizonte, Roxo 90, Grafite, Aporé and Vereda were the most efficient in BNF, when grown in the presence of urea fertilizer;

- Under controlled conditions (no limitation of temperature, water and nutrients), only with BNF and soil as sources of N for plants (without fertilization with urea), common bean genotypes cannot produce grain, shoot or root dry matter equal to those fertilized with urea.

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